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Using Synthetic Lethality to Sensitize Pediatric Brain Cancer Cells to the DNA Damaging Effects of Radiation Therapy

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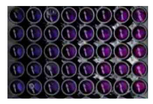
INTRODUCTION

Brain tumors are the leading cause of cancer related deaths in children. As a treatment, radiotherapy is very effective, but it also comes with many severe side effects, such as radiation-induced adverse developmental and psychosocial effects.[2] Cancer, because it is a mutagenic disease, is by definition a disease of DNA repair pathways. Here we utilize drugs to inhibit various DNA repair pathways with the purpose of sensitizing cancer cells to radiotherapy so that lower doses may be used. Hopefully this would improve the lifelong outcomes of patients that would otherwise not survive or would be left with lifelong neurocognitive problems.

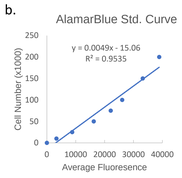
METHODS

AlamarBlue was used to determine the number of cells in each well by quantifying metabolic activity of cultured cells. The metabolically active cells reduce the blue AlamarBlue substrate into a pink product (Fig. 1a). A measure of cell kill or cell viability is determined by comparing the conversion of AlamarBlue to a standardized curve (Fig. 1b). Hydrogen peroxide was used to mimic the DNA damaging effects of radiotherapy by causing single and double stranded breaks (Fig. 3a). Various drugs were used to inhibit the repair pathways of the cells (Fig. 3b-f). Four different cell lines underwent these same experimental conditions with each inhibitor drug (Fig. 1c).

a.



b.



c.

Cell Line	Type of Cancer Cell
RES196	Childhood ependymoma
C6	Rat glioma
U118	Aggressive adult glioblastoma
H59EP	Mouse ependymoma

Figure 1. a) Image of AlamarBlue (AB) standardization. Lower part of the image shows near complete conversion of AB by metabolic activity; b) AB standardized curve allowing for the correlation between cell count and fluorescence as measured by a microplate reader. The r^2 value is .9535, indicating a good fit for the linear regression, c) Name and description of cell lines used.

RESULTS

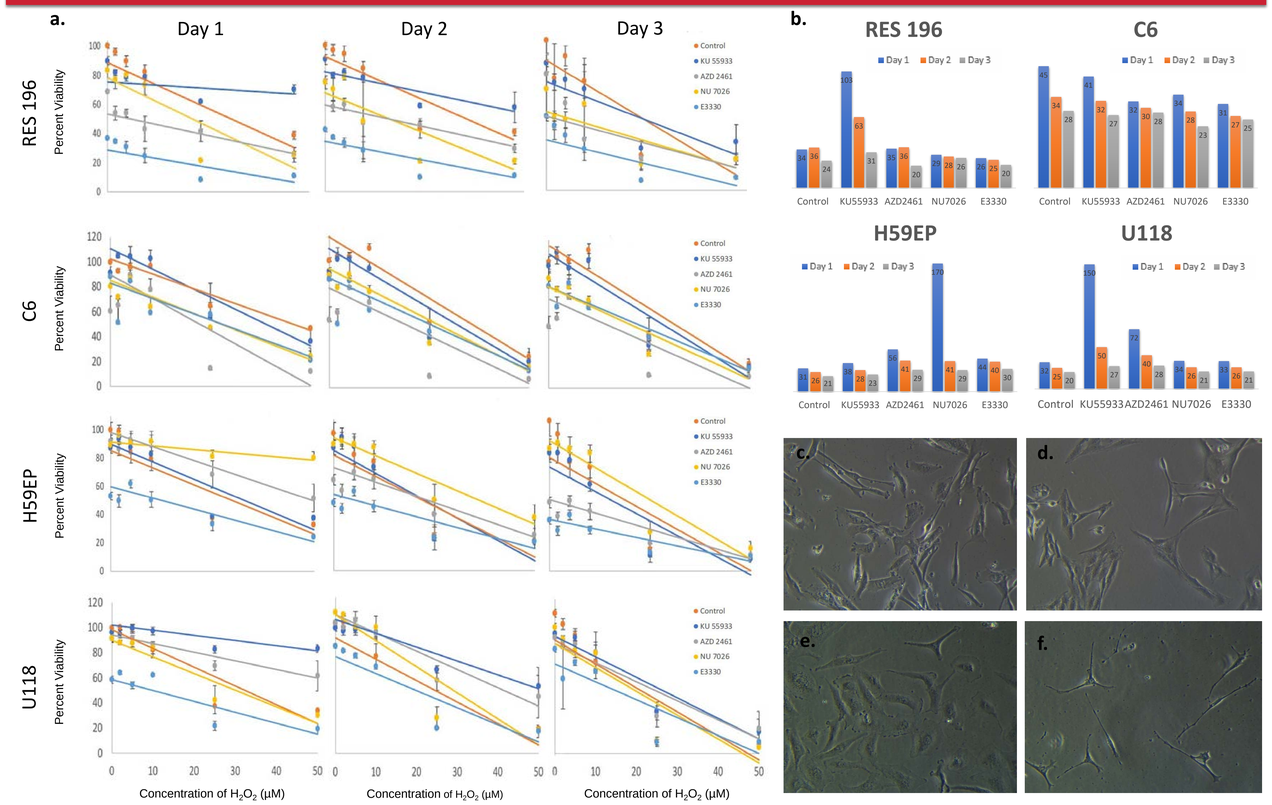


Figure 3. a) Cell viability of the different cell lines after being treated with the DNA-repair inhibiting drugs at different concentrations of hydrogen peroxide. AlamarBlue was used to measure the viability of the cells each day for three days after exposure to hydrogen peroxide, b) LD_{50} of hydrogen peroxide of control and when treated with each of the four drugs at each day, c) microscope image of U118 cell line, d) microscope image of C6 cell line, e) microscope image of RES 196 cell line, f) microscope image of H59EP cell line.

THE INHIBITOR DRUGS

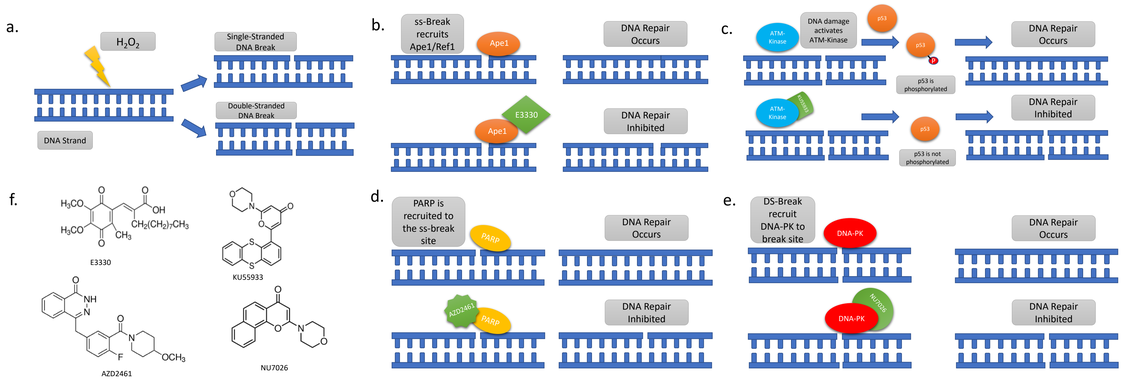
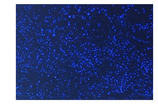


Figure 2. a) Hydrogen peroxide treatment mimics the DNA damage caused by ionizing radiation by generating single-strand and double-strand breaks. b) E3330 targets APE1/Ref-1 and is a redox inhibitor of the protein by destabilizing the protein's structure. APE1/Ref-1 is a protein that controls both DNA repair and redox reactions that allow cancer cells to thrive.[3], c) KU-55933 inhibits the ataxia-telangiectasia mutated (ATM), which is the activated by DNA double stranded breaks and initiates signaling cascades that start DNA damage response,[1] d) AZD2461 inhibits the healing of single-stranded breaks by binding to the nuclear enzyme poly(ADP-ribose) polymerase (PARP) a protein that plays a significant role in DNA repair,[6] e) NU7026 is an inhibitor of DNA-dependent protein kinase (DNA-PK), a complex that acts as a scaffold for DNA repair, and inhibits repair of double stranded breaks in cells that have the DNA-PK. NU7026 has been shown to impair cell survival,[5] f) molecular structure of the drugs used in this experiment.

CYTATION

The Cytation imager will be used in the Fall for experiments. The cells get stained with Hoechst stain, which stains the nuclei of each cell. The Cytation imager counts the actual number of cells by counting the stained nuclei instead of measuring metabolic activity, like AlamarBlue (Fig. 4a). This experiment shows that there is a linear correlation between number of cells in the well and cell counts provided by the Cytation (Fig. 4b).

a.



b.

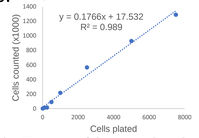


Figure 4. a) image taken by the Cytation of the stained nuclei, b) linear correlation between the average number of cells the Cytation counted versus the number plated the day before imaging.

DISCUSSION

The cancer cells saw a high toxicity with the concentrations of AZD2461 and E3330 which may have lead to some of the inconclusive data that was recovered (Fig. 2a). Lower concentrations of these two drugs will be used in future experiments, this should increase the accuracy of the LD_{50} of hydrogen peroxide. We also saw an increase in the LD_{50} of many of the cell lines when treated with NU7026 and KU55933, which may indicate that knocking out the one repair pathway actually allowed the cells to thrive rather than die (Fig. 2b). The statistical significance of this data must still be determined and more experiments must be done to provide more conclusive information. In conclusion, there may be a correlation between certain drug treatments and increased sensitivity to DNA damage similar to that caused by radiotherapy. However, more research must be done to draw more reliable conclusions.

ACKNOWLEDGEMENTS

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